

Abstract of the Disclosure

An apparatus for total internal reflection microscopy of a sample, comprising a microscope objective lens; an excitation beam path for passing light through the objective lens to said sample; and a coupling element arranged in a back focal plane of the objective lens or in a plane which is conjugate to said back focal plane; said coupling element comprising a first area for relaying light to the objective lens for total internal reflection illumination of said sample and a second area; wherein said second area is capable of separating light emitted by said sample and passing through said excitation beam path in reverse direction from said excitation beam path; wherein said second area is spatially separate from said first area and does not overlap with said first area; and wherein a distance between said optical axis of the objective lens and that boundary of said first area which is nearer to said optical axis of the objective lens is selected such that the light beams passing from said first area into the objective lens are imaged by the objective lens at angles onto said sample for which total reflection of these light beams occurs.